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Corticosterone Levels, Heterophil/Lymphocyte Ratios and Growth Rates in Lohmann Indian River Chickens Raised under Monochromatic Blue Light

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Abstract: Light intensity affects the growth rate of chickens. Previous research reported that blue light with a wavelength of 460 nm can increase the growth rate of chickens compared to green and red light. No reports have investigated whether the effects of 460-nm blue light remain even when the change in lighting is intermittent. This study therefore aimed to obtain more information about the effects of exposing chickens to 460-nm blue light with differing intensity levels. This study used roughly 2700 one-day-old Lohmann chickens raised at Wonokerto, Turi, in the Sleman District of Jogjakarta. The chickens were divided into 3 groups: a group without artificial lighting (the control), a group with intermittent blue lighting (with 12 h under monochromatic blue light) and a group with continuous monochromatic blue lighting. Blood samples were taken on days 1, 7, 14, 21 and 28. Then, the blood samples were separated into their plasma and serum components. The plasma was used to determine the numbers of heterophils and lymphocytes, while the serum was frozen to detect corticosterone levels using an enzyme-linked immunosorbent assay (ELISA). The results showed that exposure to the 460-nm wavelength blue light increased the body weights of chickens as measured at the 4th and 5th weeks. This increase started during the 3rd week ($p<0.05$). Neither intermittent nor continuous blue lighting affected corticosterone levels ($p>0.05$) except on day 14 ($p<0.05$). For the H/L ratio, blue lighting did not affect the 3 groups, except on day 7. In general, it can be concluded that blue lighting that is 460 nm in wavelength can be used in broiler chicken farms because it increases the body weights of chickens but does not increase their corticosterone or H/L ratios, both of which serve as indicators of stress.

Key words: Lohmann, corticosterone, H/L ratio, blue light

INTRODUCTION

In order to develop a profitability program useful to most broiler growers, micro environmental factors such as light and temperature recommendations are important to optimize profitability and welfare of broiler production (Olanrewaju *et al*., 2012). Lighting is an exogenous factor affecting the physiological process of growth in poultry (Olanrewaju *et al*., 2006). The most common lighting used is green light (Zhang *et al*., 2014) and blue light (Mauludin, 2014), with green being used during the early stages and blue being used at later stages of growth (Cao *et al*., 2008). In a final assignment report, Mauludin (2014) reported that the increases in the body weights of chickens raised with continuous normal, green and blue light were 1531, 1537 and, 1606 g/chicken, respectively. While previous research has been conducted using continuous lighting methods, in the interest of electrical efficiency, research is needed on the effects of intermittent lighting (12L:12D). Intermittent lighting would hopefully benefit both farmers and chickens. Indeed, it has been shown that there is no deterioration in the routine activities of birds at this level of light intensity.

In the field, the growth of chickens can be inhibited by stress due to their environment, transportation and cage conditions. In this study, corticosterone levels and ratios of heterophils and lymphocytes were measured, as both factors are stress indicators. The aim of this study was to improve the available information regarding the effects of blue lighting with a wavelength of 460 nm on the growth rate of chickens and to observe its effects on corticosterone levels and the H/L ratio.

MATERIALS AND METHODS

Time and research location: The animals used in this study were 2700 day-old chicks (DOCs). The research was conducted at a poultry farm in the village of Wonokerto, Turi, Sleman, Jogjakarta. Hormonal analyses were conducted at the Physiology Laboratory, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia. Blood examinations were performed at the Clinical Pathology Laboratory of the Faculty of Veterinary Medicine at the University of Gadjah Mada.

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A total of 2700 Lohmann DOCs were grouped randomly into 3 treatment groups: the control group, the group treated with intermittent monochromatic blue light (IBL) (12L:12D) and the group treated with continuous monochromatic blue light (CBL) (24L:0D). Each group consisted of 900 chickens. The body weights of the chickens were measured on days 1, 7, 14, 21 and 28.

Blood examination: At least 1 mL of blood was collected using a syringe and placed into a micro tube with ethylenediamine tetra acetic acid (EDTA). Blood samples were taken from the wing veins of the chickens. These examinations were conducted to count the ratio of heterophils to lymphocytes (H/L) and to measure corticosterone levels using a commercial kit DRG International, Inc., USA.

Corticosterone assay: Plasma corticosterone concentrations were determined using a commercial enzyme ELISA immunoassay kit for corticosterone (EIA-4164) (DRG, International, Inc, USA).

Statistical analysis: The collected data were subjected to statistical analyses for the interpretation of the results using an analysis of variance technique with a completely randomized design. Treatment means were compared with the Duncan Multiple Range Test (Steel *et al*., 1996).

RESULTS

Body weight gain: The body weight gains over 28 days of treatment are shown in Table 1. There were significant differences among the control, IBL and CBL groups ($p<0.05$).

Corticosterone levels: According to the homogeneity test, the cortisone levels on the 1st day were homogenous in the control, IBL and CBL groups (0, 16). Corticosterone profiles in Lohmann chickens in the early stages (day 1) of the study can be seen in Table 2. The means of the corticosterone levels were 7.88±2.28 ng/mL in the control group, 6.76±1.15 ng/mL in the IBL group and 6.75±1.07 ng/mL in the CBL group. Based on the ANOVA test, no significant difference ($p>0.05$) between the 3 groups was noted (Table 2).

On days 7, 14, 21 and 28, a homogeneity test showed that all 3 groups were homogeneous, except for the treatment data on day 14. On day 14 of the study, the data were not homogeneous and the ANOVA test results on day 14 indicated a significant difference between the 3 groups (Table 3).

Heterophil/lymphocyte ratio: Early results for H/L ratios (day 1) with the homogeneity test indicated that homogeneity was 0.38 ($p>0.05$), suggesting that the data were all homogeneous. The ANOVA test result was 0.647 ($p>0.05$), meaning that there were no differences between the 3 groups (Table 4). The homogeneity test data on day 7 indicated that the data were not homogeneous. The ANOVA test showed a result below 0.05. This meant there were no significant differences between the treatment groups. The results from day 7

Table 1: Body weight gains of chickens over 28 days in the control, IBL and CBL groups

	---------- Weight gain over 28 days (g/chicken) ----------		
	Control	IBL	CBL
1	1361.10	1510.20	1568.97
2	1365.43	1514.23	1573.63
3	1366.23	1514.60	1572.30
Average±SD	1364.92±19.71^a	1513.01±49.84^b	1571.64±23.55^c

Values (Mean±SD) with different letters differ significantly ($p<0.05$)

Table 2: Corticosterone levels in Lohmann chickens upon early examination (day 1). By day 2, the group showed non-significant results

	Corticosterone levels (ng/mL) on day-1	Test for homogeneity
Treatment	Mean±SD	0.16 ($p>0.05$)
Control	7.88±2.28^a	
IBC	6.76±1.15^a	
LBC	6.75±1.07^a	

Values (Mean±SD) with the same letters do not differ significantly ($p>0.05$)

Table 3: Corticosterone levels (ng/mL) on days 7, 14, 21 and 28

	----- Corticosterone levels (ng/mL) on experimental days -----			
Treatment Group	7	14	21	28
Control	8.69±2.41^a	8.78±2.31^a	7.63±3.16^a	6.58±0.40^a
IBL	11.66±6.28^a	6.43±1.13^b	6.63±1.10^a	6.10±2.11^a
CBL	6.37±1.19^a	5.70±1.43^c	6.73±0.87^a	4.75±0.68^a

Values (mean±SD) with the same letters do not differ significantly ($p>0.05$). On the other hand, the mean values of numbers with different letters differ significantly.

Table 4: Ratio of H/L in Lohmann chickens upon early examination (day 1). By day 2, all groups had non-significant results

	Ratio of H/L on day-1	Test of homogeneity
Treatment	Mean±SD	0.38 ($p>0.05$)
Control	1.08±0.70^a	
IBC	0.80±0.30^a	
LBC	0.92±0.29^a	

Values (mean±SD) with the same letter in each column are not significantly different ($p>0.05$).

Table 5: H/L ratios in Lohmann chickens on days 7, 14, 21 and 28

	---------- Ratio H/L on experimental days ----------			
Treatment Group	7	14	21	28
Control	1.05±0.74^a	0.74±0.52^a	0.40±0.18^a	0.77±0.36^a
IBL	0.49±0.12^b	0.64±0.33^a	0.48±0.13^a	0.43±0.14^a
CBL	0.32±0.14^c	0.83±0.20^a	0.64±0.21^a	0.72±0.29^a

Values (Mean±SD) with the same letter in a column do not differ significantly ($p>0.05$). On the other hand, mean values with different letters differ significantly.

were not the same as those from days 14, 21 and 28, for which the results were homogeneous but did differ significantly between the 3 groups (Table 5).

DISCUSSION

Lighting is an important factor influencing the growth process of broiler chickens. In this study, it was found that both intermittent and continuous blue light with a wavelength of 460 nm can increase the body weights of chickens ($p<0.05$), starting on day 21 until day 28 (Table 1) (Fig. 1). These results are in accordance with the results of Pan *et al.* (2014), who reported that artificial polychromatic light affects the growth and physiology of chickens. Halevy *et al.* (1998), Rozenboim *et al.* (1999) reported the blue color, have the same effects include violet, green and red light (Kram *et al.*, 2010). The lighting frequency and method also affected the growth rate, as has been reported by previous researchers (Cao *et al.*, 2008). Indeed, Cao *et al.* (2008) reported that monochromatic LED lighting increases the growth of broiler chickens reared under green monochromatic light during the early stages of growth by enhancing the proliferation of skeletal muscle satellite cells. Similarly, Mahmood *et al.* (2014) reported that variations in light intensity conditions exert effects on the growth rate, health and behavior of broiler chickens.

The high body weights of the chickens that received lighting treatment were likely attributable to the lower activity levels of the chickens, leading to a conservation of energy and a corresponding weight gain. This finding is in accordance with previous studies, which stated that broilers reared at 5 lux of light spent more time in sleeping and less time in preening and foraging than those reared under 50 and 200 lux treatments (Alvino *et al.*, 2009). Aggressive behavior and pecking activity were significantly higher in the birds reared under high light intensity (50 lux) (Mohammed *et al.*, 2010).

Importantly, birds, poultry included, can perceive light in different, non-pictorial ways. Birds possess three key photosensitive areas: (1) the retina, which absorbs photons by photo-pigments rhodopsin (rods), iodopsin (cones) and melanopsin, (2) the pineal gland and (3) the hypothalamus (Anonymous, 2014). These 3 areas play a very important role in biological and physiological functions (Suwindra and Balnave, 1986; Apeldoorn *et al.*, 1999; Hartwig and van Veen, 1979; Hatori and Panda, 2010).

Insulin-like growth factors and the thyrotrophic [triiodothyronine (T3) and thyroxine (T4)] axis are considered to be prerequisites for normal growth and development (Decuypere and Buyse, 2005).

Unfortunately, stress can suppress the growth rates of animals. The measurement of corticosterone levels as a stress indicator in animals has been validated by Lentfer *et al.* (2015) and Matur *et al.* (2015). In house finches, plasma levels of glucocorticoids can increase

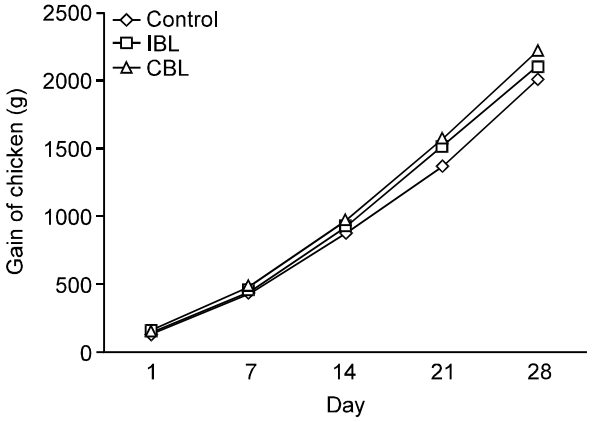

Fig. 1: Body weight gains over 28 days. Different body weight gains started on day 21

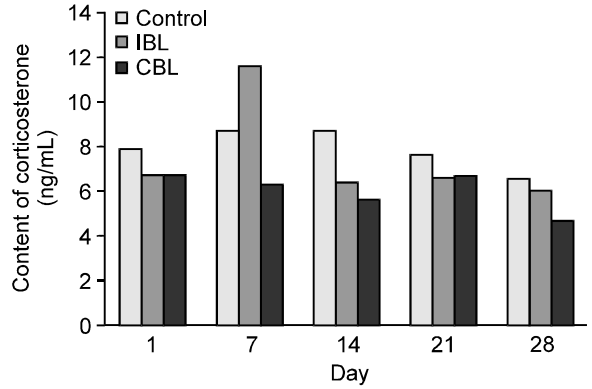

Fig. 2: Corticosterone levels in the 3 groups (control, IBL and CBL) of chickens

by a full order of magnitude within 3 min of capture (Romero and Romero, 2002; Muller *et al.*, 2011) until 20-30 min after capture. This suggests that handling time should be very short. In contrast, the H/L-ratio remains constant for at least 1 h after capture in house finches (Carpodacus mexicanus; Davis *et al.*, 2008), permitting a longer time frame to obtain blood samples. Romero and Remage-Healey (2000) reported that starlings have daily rhythms in both basal corticosterone levels and in their response to stress, with more corticosterone released during the night in response to identical stimuli.

Elevation of corticosterone, the major glucocorticoid in birds, leads to an increase in the short-term survival mobilization of energy reserves (Wingfield *et al.*, 1998). In general, exposure to continuous and intermittent blue light does not result in any significant differences in corticosterone levels ($p>0.05$) (Fig. 2). Even on day 14, there was a difference with the corticosterone levels in the CBL and IBL groups, which were lower than those of the control group ($p<0.05$). It can therefore be concluded that blue light does not elevate corticosterone levels and

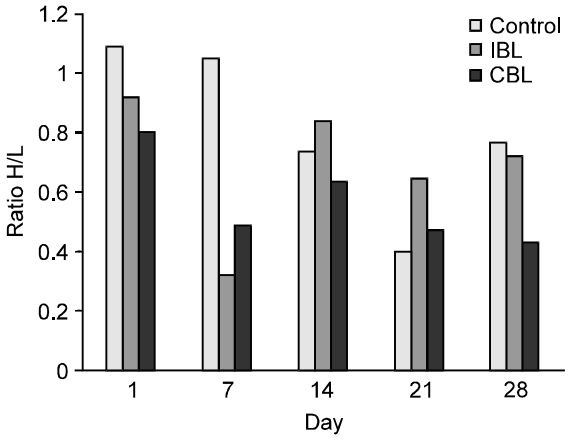

Fig. 3: H/L ratios of the 3 groups (control, IBL, CBL) of chickens

thus does not induce undue stress on the chickens. High corticosterone levels in the animals were likely caused by stress during transportation, lighting changes, cage movement, blood sampling and appetite decrease. This was in accordance with the findings of Muller *et al.* (2011), who reported that baseline corticosterone concentrations were only elevated when the animals had no body fat stores and as a reaction to the presence of researchers at the nest.

Another indicator of stress is the ratio of heterophils to lymphocytes in the blood (Gross and Siegel, 1986). Number of leucocyte increase during mildly or moderately stressful conditions and consequently the heterophil/lymphocyte ratio can be used to detect the presence of physiological stress for most stressors. (Maxwell and Robertson, 1998). On the other hands, it appears that baseline corticosterone concentration and H/L-ratio differ in sensitivity to various stressors (Claudia *et al.*, 2011). The H/L ratio was found to be approximately 0.6 in normal gulls and this increased to over 2.9 in gulls that were oiled, emaciated, infected with endoparasites, or injured. In the current study, the H/L ratio was very high on day 1, rising from 0.8 to 1.08. This finding was likely attributable to the transportation of the newly arrived DOCs, which had just traveled hundreds of miles and were therefore expected to be in a highly stressed state. The H/L ratio decreased over the next day as the animals became adapted to their new environment ($p>0.05$) (Fig. 3), except on day 7 ($p<0.05$). Upon further observation, it appeared that adaptation in the animals started on treatment day 7, where it could clearly be seen that the H/L ratios in the treatment groups decreased while in the control group the ratio remained relatively the same. This finding suggests that continuous or intermittent blue light did not elevate the H/L ratios and therefore did not increase the stress levels of the animals. Matur *et al.* (2015) reported the main effects of social stress were reflected in the heterophil and lymphocyte percentages and the H/L ratio. Under stress conditions, the heterophil percentage is higher, the H/L ratio is higher and lymphocyte percentage is lower.

It can be assumed that corticosterone directly causes a proportional change in the H/L-ratio and that this reflects the levels of circulating corticosterone over time (Muller *et al.*, 2011). This study could not determine if corticosterone causes the same results as a previous study because the corticosterone profile was not found to be similar to the H/L ratio. Lentfer *et al.* (2015) also found that population-based reference values may not be sensitive enough to detect individual stress reactions and that the H/L ratio as an indicator of stress under commercial conditions may not be useful because of confounding factors. They thus suggest that other non-invasive measurements of stress should be adopted.

Conclusions: Based this study, it can be concluded that intermittent or continuous monochromatic blue light with a wavelength of 460 nm can increase a chicken's body weight ($p<0.05$) but does not increase corticosterone levels ($p>0.05$) or the H/L ratio ($p>0.05$), both of which are indicators of stress. Thus, blue lighting with a wavelength of 460 nm is useful for increasing body weight in a commercial context.

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